Nemaline Myopathy is the most common non-dystrophic congenital myopathy, clinically characterized by muscle weakness. The disease is associated with mutations in the nebulin gene and the nebulin-based disease is referred to as NEM2. Recent work on skinned muscle fibres from NEM2 patients revealed remarkable phenotypic similarities to fibres from nebulin KO mice (Ottenheijm et al, 2012). Here we investigated mechanics and kinetics of single myofibrils from a novel NEM2 mouse model (NEB Δex55) that mimics a deletion in the nebulin gene found in a large group of NEM2 patients. We used rapid solution switching (Tesi et al.,2002) to compare maximal tension and kinetics of contraction and relaxation of myofibrils isolated from frozen skeletal muscles (tibialis cranialis of neonatal mice) of WT and NEB Δex55 mice. Myofibrils, mounted in a force recording apparatus (15 °C), were maximally $Ca^{2+}$-activated (pCa 4.5) and fully relaxed (pCa 9.0). Maximal isometric tension was markedly reduced in NEB Δex55 mouse myofibrils ($49.7 \pm 10.6$ mN $mm^{-2}$n=11) compared to WT ($135.3 \pm 16.9$mN $mm^{-2}$n=9).The rate constant of active tension generation following maximal $Ca^{2+}$ activation ($k_{ACT}$) was significantly reduced inNEB Δex55 mouse myofibrils ($1.46 \pm 0.07s^{-1}$) compared to WT ($2.75 \pm 0.27$ $s^{-1}$). Force relaxation kinetics was remarkably faster in NEB Δex55 mouse myofibrils than in WT, evidence that the apparent rate with which cross-bridges leave the force generating states is accelerated in the NEB Δex55sarcomeres. Reduction of the rate with which cross-bridges enter force generating states and of cross bridge dissociation can markedly contribute to reducing maximal tension. This is expected to increase the energetic cost of tension generation of the NEB Δex55sarcomeres. Results suggest that nebulin plays a significant role in contraction regulation and that altered cross bridge kinetics contribute to NEM2 pathogenesis

**2473-Pos Board B492**

**Increased Fatigue Resistance of Skeletal Muscle with Elevated 2-Deoxy-ATP following Ribonucleotide Reductase Overexpression**

**Jacqueline N. Robinson-Hamm**[1], Sarah G. Nowakowski[1], Michael P. Siegel[1], Rachel M. Faber[1], Robert S. Weiss[2], Glen B. Banks[1], David J. Marcinek[1], Michael Regnier[1].
[1]University of Washington, Seattle, WA, USA, [2]Cornell University, Ithaca, NY, USA.

Skeletal muscle myosin can use a variety of nucleotides to varying effectiveness as substrates for contraction. We previously demonstrated that complete replacement of ATP with 2 deoxy-ATP (dATP) in activation solutions increases contraction of demembranated rabbit skeletal muscle and that enhanced performance results from increased myosin binding and cycling kinetics, especially during sub-maximal calcium activation. Here we report on a transgenic mouse (Tg-RR) that overexpresses the enzyme, ribonucleotide reductase, which converts ADP to dADP (that is rapidly phosphorylated to dATP). This results in a ~10x increase in dATP content of skeletal muscle, which still constitutes $\leq$ 1% of the adenosine triphosphate nucleotide pool. We are examining the contractile and metabolic properties of skeletal muscle in this transgenic model. Preliminary data indicates the phosphocreatine to ATP (PCr:ATP) ratio of Tg-RR mice is significantly elevated relative to wild type (Tg-WT) mice, suggesting the Tg-RR mice may have an energetic advantage due to increased high energy phosphate reserves. Furthermore, the Tg-RR mice ran for a longer period of time at increased speed and for longer distances in a graded exercise test. Direct *in situ* stimulation of the gastrocnemius muscle indicates improved resistance to fatigue, consistent with both the exercise and the metabolic (PCr:ATP) findings. Interestingly, our preliminary experiments suggest the Tg-RR mice produce significantly less peak force and show signs of atrophy. Ongoing experiments are examining changes in mitochondrial content and function as well as contractile properties of isolated muscles to determine the mechanisms underlying the increased fatigue resistance of the Tg-RR mice. These results suggest a new direction for developing interventions to improve exercise tolerance in human patients. Supported by HL11197 (MR).

**2474-Pos Board B493**

**Crossbridge Properties during Fatigue and Recovery in Mouse Skeletal Muscle Fibres**

**Giovanni Cecchi**, Marta Nocella, Barbara Colombini, Maria Angela Bagni.
University of Florence, Firenze, Italy.

Fatigue occurring during exercise can be defined as the inability to maintain the initial force or power output. We showed recently that fatigue during repetitive tetanic stimulation at 24 °C of fibre bundles dissected from FDB mouse (C57BL/6) muscle, occurs in two phases: an initial one during which individual crossbridge force decreases, and a later phase during which also crossbridge number decrease (Nocella et al.,2011 J Physiol 589).The present experiments were made on the same preparation to compare the fatigue mechanism at 24°C and at physiological temperature of 35 °C and to investigate the mechanism of force recovery from fatigue at 24°C. Fatigue was induced with 105 consecutive isometric tetani evoked every 1.5 s. Force recovery was followed by tetani evoked every 90s until force recovered to 90-100% of pre-fatigue value. Stiffness was measured with small sinusoidal length oscillations at 6.5 kHz. At both temperatures fatigue occurred initially through the decrease of the individual crossbridge force followed by the reduction of crossbridge number. However the initial phase lasted for ~40 tetani at 35°C and ~20 tetani at 24°C. This suggests a greater resistance to fatigue of this mechanism at high temperature. In contrast, during the second phase the tension loss was faster at 35°C than at 24°C so that after 105 tetani tension was similar at both temperatures. Force recovery also occurred in two phases. The initial phase lasted from ~1.5-4 min and recovered 40-90% of tension loss. The second phase lasted for ~60 min and its amplitude was well correlated with tension decrease during the second phase of fatigue. Thus the mechanism of tension recovery after fatigue seems symmetrical to tension loss during fatigue.

**2475-Pos Board B494**

**Localization and Function of Xinα in Mouse Skeletal Muscle**

**Hanzhong Feng**[1], Qinchuan Wang[2], Jenny L.-C. Lin[2], Jim J.-C. Lin[2], Jian-Ping Jin[1].
[1]Department of Physiology, Wayne State University School of Medicine, Detroit, MI, USA, [2]Department of Biology, University of Iowa, Iowa City, IA, USA.

Xin repeat-containing proteins were originally found in the intercalated discs of cardiac muscle with proposed roles in cardiac development and function. A pair of paralogous genes, Xinα (Xirp1) and Xinβ (Xirp2), is present in mammals. Ablation of the mouse Xinα (mXinα) did not affect heart development but caused late-onset adulthood cardiac hypertrophy and cardiomyopathy with conductive defects. Both mXinα and mXinβ are also found in the myotendinous junctions (MTJs) of skeletal muscle. In the present study, we investigated the structural and functional significance of mXinα in skeletal muscles. In addition to MTJs and the contact sites between muscle and perimysium, mXinα but not mXinβ was found in the blood vessel walls, whereas both proteins were absent in neuromuscular junctions or the nerve fascicles. Co-localization and co-immunoprecipitation suggested association of mXinα with talin, vinculin and filamin but not β-catenin in MTJs of adult skeletal muscle. Complete loss of mXinα in mXinα-null mice had subtle effects on the MTJ structure and the expression of other known MTJ components. Diaphragm muscle fibers of mXinα-null mice showed significant hypertrophy. In comparison with wild type controls, mouse extensor digitorum longus (EDL) muscle lacking mXinα exhibited no overt change in contraction and relaxation velocities or in maximum force development. Its fatigability and recovery from fatigue were similar to that of wild type control. Loaded fatigue contractions generated stretch injury in wild type EDL muscle as indicated by an adaptive restrictive truncation of troponin T. However, this effect was blunted in mXinα-null EDL muscle. The results suggest that mXinα may play a role in MTJ conductance of contractile and stretching forces, essential to skeletal muscle function.

**2476-Pos Board B495**

**Cytoskeletal Tension differences between Normal and Dystrophic Myotubes Probed with FRET Based Stress Sensors**

**Thomas M. Suchyna**, Fanjie Meng, Frederick Sachs.
SUNY at Buffalo, Buffalo, NY, USA.

Duchene muscular dystrophy is caused by the loss of the cortical cytoskeletal protein dystrophin. These muscle cells have abnormally structured cortical cytoskeleton that leads to impaired ability to control stress in the cell cortex. When a stress bearing cytoskeletal element is removed from a system other proteins rearrange to adapt to the changed stress distribution and must absorb stresses that they were not intended to bear. This could affect a number of downstream mechanically sensitive receptors and enzymes. Knowing which cytoskeletal elements absorb the stress in the system that was intended for dystophin would be useful in understanding what mechanically based sensory systems will be affected most and can help in the design of treatments and in assessing therapies to treat muscular dystrophy. We created chimeric cytoskeletal proteins containing the cpstFRET stress sensing cassette and expressed them in developing normal and dystrophic mouse myotubes. These proteins included actinin, filamin, spectrin, vinculin and dystrophin. These chimeric proteins all showed distinct spatial distributions in the myotubes. We measured the stress on these proteins in resting cells and in cells stretched with a micropipette. All proteins had different resting stress levels. Filamin, an important component of focal adhesion plaques, showed the most significant difference in resting stress levels between normal and dystrophic myotubes. It also showed